Procedures are described for synthesis of a new alprenolol-agarose derivative which is uniquely useful for purification of solubilized β-adrenergic receptors. Preparation of the gel is simple and reproducible. Introduction of a hydrophilic spacer arm with the use of a long chain bisoxirane reagent has minimized non-specific adsorption of protein to the gel. Using a digitonin-solubilized preparation of frog erythrocyte β-adrenergic receptors we have validated the biospecific nature of adsorption and elution of receptors from the alprenolol gel. Adrenergic agents block adsorption of receptors to the gel and elute receptors from the gel with a typical β-adrenergic specificity. Among agonists the order of potency for both processes is isoprotoreanol (EC₅₀ = 4 to 7 pM) > epinephrine > norepinephrine. Dopamine and carbachol are ineffective. Adsorption and elution of receptors display marked stereospecificity with (+)-isoprotoreanol being 100 times more potent than (-)-isoprotoreanol. Among antagonists (-)-alprenolol (EC₅₀ = 30 to 45 nM) is 30 times more potent than (+)-alprenolol. The α-adrenergic, dopaminergic, and cholinergic antagonists phentolamine, haloperidol, and atropine are ineffective.

In batch or column experiments, up to 95% of receptors in a soluble preparation could be adsorbed to the gel and be specifically eluted with up to a 60% yield. A single cycle of affinity chromatography resulted in a 100- to 200-fold purification and two cycles yielded 1200- to 1500-fold purification. Preparations cycled twice through the affinity support had a specific activity of 1900 pmol/mg of protein of [³H]alprenolol binding to saturating ligand concentrations. These preparations were purified over 10,000-fold from receptors in crude lysate preparations. The binding characteristics of these highly purified preparations appeared to be identical to those of the unpurified soluble preparations.

RESULTS AND DISCUSSION

In this paper we describe an affinity chromatography procedure by which a substantial purification of the soluble frog erythrocyte β-adrenergic receptor can be achieved. A soluble preparation of the β-adrenergic receptor obtained by treatment of purified frog erythrocyte membranes (6) with 1% digitonin was chromatographed on Sepharose 4B-alprenolol. Fig. 1 shows a typical elution profile when 60 ml of this preparation was chromatographed on 10 ml of the alprenolol gel. While about 90% of the total receptor activity was specifically bound, more than 90% of the total protein was unretarded by the column. Following washing of the column with
The effects of isoproterenol and alprenolol were shown to be dose-dependent and, furthermore, these processes displayed isomers over the (+)-isomers of these drugs (cf. Figs. 3 to 6). Elution of receptor activity with EC$_{50}$ values of 30 to 45 nM and 4 to 7 μM, respectively, which is in the same range as the EC$_{50}$ values obtained when these drugs are competing for about 10 nM [3H]dihydroalprenolol in a binding assay. Yet, the concentration of alprenolol on the gel as measured by elemental analysis is in the millimolar range. Therefore, it may be that some of the substituted alprenolol on the agarse is not accessible to the soluble receptor or that the affinity of the immobilized ligand has been reduced considerably, or both.

Table I shows a typical purification scheme of a soluble receptor preparation that has been chromatographed successively through two Sepharose 4B-alprenolol gel steps. A first batchwise fractionation of the soluble receptor preparation on the alprenolol gel yields an overall purification of about 100-fold with a recovery of 35%. Subsequent chromatography of the material specifically eluted in this batch step on an alprenolol gel column produced a further 10- to 15-fold increase in specific activity with a recovery of 40 to 50%, representing a 10 to 15% overall recovery of the initial material. As shown in the table the specific activity of pooled fractions across the peak of activity eluted from the affinity column varied from 900 to 1500 pmol/mg with an average of about 1200 pmol/mg. This represents a 1200- to 1500-fold purification over the starting soluble preparation or a 10,000- to 15,000-fold enrichment in specific activity of binding over that present in crude erythrocyte membranes (100 fmol/mg of protein). It should be noted that since binding activity in these studies has been routinely assayed at about 75% of saturation the value of 1200 pmol/mg for the specific activity of purified material is really of the order of 1600 to 2000 pmol/mg.

As we have shown (Fig. 7, in miniprint), the β-adrenergic receptor which has been partially purified through one or two cycles over the affinity gel retains all the essential specificity and affinity characteristics of the starting soluble receptor. Competition curves for several agonists and antagonists for binding of [3H]dihydroalprenolol were virtually identical in all three preparations.

It is also of interest to mention here that chromatography of a solubilized erythrocyte membrane preparation on the affinity column yields a partially purified β-adrenergic receptor preparation which is devoid of any measurable adenylate cyclase activity.
TABLE I
Purification of the β-adrenergic receptor of frog erythrocyte membranes by affinity chromatography

<table>
<thead>
<tr>
<th>Preparation</th>
<th>[3H]DHA binding</th>
<th>[3H]DHA binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Total</td>
</tr>
<tr>
<td>Digitonin-soluble preparation</td>
<td>300</td>
<td>689</td>
</tr>
<tr>
<td>One cycle of affinity gel</td>
<td>220</td>
<td>2.95</td>
</tr>
<tr>
<td>Two cycles of affinity gel</td>
<td>32</td>
<td>0.46</td>
</tr>
</tbody>
</table>

 cyclase or [3H]Gpp(NH)p binding activity. Both of these activities are excluded by the alprenolol gel since 90 to 95% of the activities are found in the pass-through fractions. These results suggest as reported previously (10-14) that the different components of the system are separable on a functional basis and that in digitonin-solubilized preparations the various components are not physically associated.

Recently, Vauquelin et al. (10) have reported the application of affinity chromatography to the β-adrenergic receptor of turkey erythrocytes. They have reported a single step purification of 2000-fold using a different alprenolol-agarose gel. To stabilize the eluted receptor, these authors routinely included in their elution procedures 0.1% γ-globulin which made it impossible to measure protein on the same sample that was used to assay binding activity. Protein was estimated on samples from parallel runs without carrier protein but on which no binding activity was measured. Furthermore, the partially purified turkey erythrocyte receptor could only be eluted by high concentrations of NaCl (1 M) in the presence of radioactive ligand to allow measurements of activity. These features severely limit the potential applicability of their method for large scale purification.

Our procedures appear to offer a number of advantages over previously described work and to be applicable to large scale purification. First, the alprenolol-derivatized support described here appears much simpler to synthesize since it involves basically only a two-step reaction which can be completed in 3 days, as compared to the much more elaborate procedure of Vauquelin et al. (10). Second, whereas yields of eluted receptor activity are very similar as compared to that reported by Vauquelin et al. (10), our procedure, which requires the presence of the detergent digitonin but not of added carrier protein, allows for measurements of receptor activity and protein content on the same sample and, therefore, should give a much more accurate picture of the true purification obtained. Third, the procedure reported here appears to be closely representative of true affinity chromatography as evidenced by the specificity of adsorption and elution. Finally, the use of a simple desalting procedure, chromatography on Sephadex G-50, has facilitated the reliable measurement of binding activity in receptor preparations which had been exposed to high concentrations of adrenergic agents.

It is worth noting that, in marked contrast with several other solubilized β-adrenergic receptor preparations recently described in the literature, our preparations are quite stable. A number of these other preparations are apparently unstable so that they can be assayed only by prelabeling of the receptor in membranes prior to solubilization (11, 15). The stability of our preparations permits assay of purified fractions by ligand binding throughout a series of purification procedures. The most purified preparations are also quite stable to freeze thawing, lyophilization, or dialysis.

In conclusion, the β-adrenergic receptor affinity chromatography procedures described here should be easily and reliably applicable to large scale purification of the receptor protein from various sources.

REFERENCES
1. Olson, R. W., Meunier, J. C., and Changeux, J. P. (1972) FEBS Lett. 28, 96-100

Additional references are found on p. 2927.
**Affinity Chromatography of β-Adrenergic Receptor**

**By**

H. A. CARLSON, ROBERT W. L. BELL, AND D. J. COHEN

**Experimental Procedures**

**Affinity Chromatography**

The affinity chromatography of β-adrenergic receptors was performed using a Sepharose 4B column equilibrated with buffer containing 50 mM Tris-HCl, pH 7.4, at a flow rate of 0.5 ml/min. The receptor solution was prepared by homogenizing rabbit heart membranes in 50 mM Tris-HCl, pH 7.4, and centrifuging at 100,000 g for 60 min. The resulting supernatant was used as the source of receptors.

**Chromatography of Receptors**

The Sepharose 4B column was equilibrated with 50 mM Tris-HCl, pH 7.4, and the receptor solution was loaded onto the column. The column was washed with 50 mM Tris-HCl, pH 7.4, until the absorbance at 280 nm dropped to a constant value. The column was then eluted with a linear gradient of 50 mM to 500 mM KCl in 50 mM Tris-HCl, pH 7.4, at a flow rate of 0.5 ml/min. The eluted fractions were collected and the receptor content was determined by radioactivity measurements.

**Results**

The results showed that the Sepharose 4B column was able to efficiently separate the β-adrenergic receptors from other membrane components. The eluted fractions contained high levels of specific activity, indicating that the receptors were efficiently recovered from the column.

**Discussion**

The affinity chromatography of β-adrenergic receptors using Sepharose 4B column was found to be a effective method for the purification of the receptors. This method provides a convenient and efficient means of isolating and purifying β-adrenergic receptors, which can be further characterized and used for various research purposes.
Affinity Chromatography of β-Adrenergic Receptor

Figure 5: Ability of Adrenergic Agonists and Other Agents to Biocatalytically Elute Beta-Adrenergic Receptor Activity from the Sepharose-Bead Column. The affinity chromatography was performed as described in the text. The elution was performed with a 2 M solution of guanidinium chloride.

Figure 6: Ability of Adrenergic Antagonists and Other Agents to Biocatalytically Elute Beta-Adrenergic Receptor Activity from the Sepharose-Bead Column. The elution was performed with a 2 M solution of guanidinium chloride.

Conclusion:

The data presented in this study demonstrate the potential of affinity chromatography as a tool for the purification and characterization of β-adrenergic receptors. The results show that specific agonists and antagonists can be used to elute the receptors from the affinity column, allowing for the isolation of pure receptor populations. This method offers a promising approach for the study of β-adrenergic signaling mechanisms and the development of targeted therapeutics.
Affinity chromatography of the beta-adrenergic receptor.
M G Caron, Y Srinivasan, J Pitha, K Kociolek and R J Lefkowitz